

Control of broad-leaved docks by *Armillaria mellea*

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Abstract. Although several plant pathogens can infect dock leaves, none of these can infect the roots and kill the plants. The starchy tap-roots of broad-leaved docks (*Rumex obtusifolius*) are extensively rotted by honey fungus (*Armillaria mellea*), the isolate tested was also pathogenic to strawberry and potato tubers, as well as a variety of woody hosts. An isolate of *Armillaria ostoyae* was also effective.

Introduction

Armillaria mellea (Vahl:Fr.) Kummer is well known as a root-rot pathogen of many species of woody plants worldwide (Rhoads 1956; Raabe 1962a, 1979; Hall *et al.* 1971; Rishbeth 1982; Greig and Strouts 1983). *Armillaria mellea* also rots several starchy-rooted plants such as strawberry (Fox and Popoola 1990), rhubarb, mint, carrot, tomato, iris (Moore 1959) and cassava (Popoola 1991), as well as potato tubers (Lea 1909; Wilson 1921), but it has not previously been reported from the perennating swollen tap-roots of broad-leaved dock (*Rumex obtusifolius* L.). *Armillaria ostoyae* (Romagnesi) Herink is usually associated with the white rot of conifers (Kile *et al.* 1991). The aim of this investigation was to test whether an isolate of *A. mellea* and an isolate of *A. ostoyae* could infect and control broad-leaved dock plants.

Materials and methods

The pathogen

The late Dr J. Rishbeth, Cambridge University, isolated the *A. mellea* and *A. ostoyae* isolates used for the tests. The identity of these English isolates was confirmed by J-J. Guillaumin and C. Mohammed at INRA Clermont Ferrand, France, using interfertility testing. The pathogenicity of the isolate of *A. mellea* had been successfully tested earlier for its virulence to potted strawberry, three-year-old bushes of privet, black currant, Lawson cypress and two-year-old seedlings of horse chestnut, using infected hazelwood billets (Popoola 1991).

Preparation of inoculum

Billet inoculum was prepared using 7-cm lengths

approximately 2.5 cm in diameter cut from straight, living, coppiced branches of hazelwood in November 1991. The billets were then sterilized by autoclaving at 121°C for 50 min in 500 ml glass jars. They were then packed aseptically onto a two-week-old culture of *A. mellea* or *A. ostoyae* growing in approximately 1.5 cm of 3% Oxoid Malt Extract Agar (MEA) in a one-litre, glass jar, using a long pair of forceps. The jars had been inoculated by transferring seven-day-old cultures growing on 3% MEA in Petri dishes. They were incubated in the dark at 25(±2)°C for two months until mycelium had grown through and over the billets colonizing the full length of the wood. This technique is more rapid than the methods described (Rishbeth 1982, 1984) for the preparation of wood inoculum.

The host material

Seeds of the broad-leaved dock (*R. obtusifolius*) were collected at the University of Reading Experimental Field Unit at Shinfield in Berkshire in the summer of 1991 and stored in a dry state at ambient room temperature. In November 1991, seeds were immersed in tap water and placed in a controlled environment cabinet with a 15°C temperature to encourage germination. Two weeks later, seedlings at the first-leaf-emergence stage (Lutman and Tucker 1987) were transferred to multi-modular seed trays containing John Innes Number 2 compost and Hoagland's Solution was applied weekly to encourage tap-root growth. After another four weeks, eight individual seedlings per treatment were transplanted into 8x8x6 cm pots containing the compost.

Pathogenicity tests with broad-leaved docks

In early February 1992, the billets inoculated with *A. mellea* were half-buried in the pots with the

seedlings, bringing the roots of the seedling and the billets into contact with each other. Control plants without billets were also replicated eight times and arranged alternately in a 4x4 square. The pots were kept in the glasshouse and watered from above, daily, on a flooded bench to prevent excess drainage of the soil. Hoagland's solution was applied monthly.

The foliar symptoms were recorded monthly and at the end of the six-month inoculation period, the roots of the broad-leaved docks were carefully removed from the soil. The viability of any mycelial fans in the roots and small pieces of infected root tissue was authenticated by plating them out onto 3% MEA containing the antibiotics oxytetracycline (Terramycin) and streptomycin sulphate (Streptomycin), at 100 mg/ml to agar. Fungal and bacterial contamination of the *A. mellea* re-isolated from rotted dock roots was prevented by repeated sub-culturing on the antibiotic-amended media every two days. After 10 days *A. mellea* was successfully isolated into pure culture.

In a subsequent experiment, three-month-old seedlings at the four-leaf stage in pots (14 cm diameter, 18 cm high, John Innes 2) were inoculated by burying the infected billets in the soil next to the dock roots to reduce the effects of air drying. Some of the pots were placed in pot saucers, while the rest were allowed to drain. There were ten replicates per treatment arranged randomly. All the pots were watered every second day. The plants were removed from the pots and assessed for decay after seven months.

In December 1992, 13 adult *R. obtusifolius* selected from a dense population of *R. obtusifolius* with some *R. crispus* and their hybrids at the University of Reading Experimental Field Unit at Shinfield, were either inoculated with *A. mellea* or *A. ostoyae*, or left uninoculated as the control. The billets were fully buried in the soil in contact with the roots. Their positions were marked with coloured pegs. In November 1993 the plants were dug up and root damage was assessed and recorded along with their dry-weights.

Results

Infection of broad-leaved docks

Despite watering daily, causing waterlogging of the soil, the portion of the billets exposed to the air dried out and there were no visible effects on the foliage of the seedlings during the first three months. In the fourth month, the older leaves of plants in two of the

inoculated pots showed signs of early senescence, becoming brown and crisp. This symptom spread to the younger leaves, until both plants ceased to produce further foliage in the fifth month. Dissection of these plants revealed the upper tap-root and crown to be dead and brown, though lower down the roots still appeared white and living. Hughes (1993) showed that buried roots of broad-leaved dock may appear healthy and new root-growth may occur for up to two months after decapitation of the crown, but without this crown the regeneration of new leaves does not occur and the roots will eventually die. Transverse slices of the dead and 'living' parts of the roots revealed what appeared to be mycelial growth of *A. mellea* within the roots. In the 'living' roots the mycelial growth was surrounded by a light-brown necrosis of the tissue. Examination of the remaining six inoculated pots revealed four of the seedlings had infected roots although there were no visible foliar symptoms. No visible trace of *A. mellea* was found in the roots of the other two inoculated pots or in any of the uninoculated control plants.

In the second experiment, the first plant died after a month. The majority of the plants infected by either pathogens had died and the roots of all plants were infected after seven months. The root dry-weights were reduced by 90%. The waterlogging of the potting compost had no significant influence on the development of the disease.

In the field experiment, both species of *Armillaria* reduced the root dry-weight of adult *R. obtusifolius* plants ($P < 0.001$). All the plants were infected by *A. mellea* apart from one. At the final harvest, five plants inoculated with *A. mellea* were dead and two more were heavily colonized. Despite this, nearly all of the plants had managed to set seed. Although all of the plants inoculated with *A. ostoyae* became severely infected by the time of harvest and loss of root dry-weight was similar to that in plants infected by *A. mellea*, only two were killed (Table 1).

Discussion

Several fungi have been reported to infect *R. obtusifolius* (Ellis and Ellis 1985; Boerema *et al.* 1980) including *Puccinia phragmites* (Schum) Körn., *Uromyces rumicis* (Schum) Wint., *Ramularia rubella* (Bon) Nannf., *Venturia rumicis* (Des.) Wint., *Phoma rumicola* sp. nov., as well as a number of common plurivorous species. A few plant pathogenic bacteria are also able to rot the roots of dock plants (Hughes

Table 1. The effect of infection by *Armillaria mellea* and *A. ostoyae* on the root dry-weight of *R. obtusifolius*.

Pathogen	Root dry-weight (g/plant)	Standard error
<i>A. mellea</i>	9.56	± 3.696
<i>A. ostoyae</i>	10.66	± 3.545
Uninoculated control	27.139	± 4.786

1993). None of these bacteria was as virulent as *A. mellea* or *A. ostoyae* which can kill *R. obtusifolius* under the correct conditions. Nonetheless, there have been no previous reports that *Armillaria* spp. can even infect *R. obtusifolius*. This may be due to oversight or because broad-leaved docks are generally not found in woodland where *Armillaria* species are common, but instead infest rough grassland and improved pasture used for dairy farming or disturbed man-made and natural environments, such as construction sites and shingle beaches.

Although meadow grasses are not susceptible, it is possible that other starchy-rooted non-target plants might also be infected by *Armillaria* species. This is unlikely to become serious unless they form extensive patches like *R. obtusifolius*. Areas rich in susceptible herbaceous plants could be isolated by a protective, cultivated or grassy strip to prevent the unwanted spread of rhizomorphs of the *Armillaria* species. This should be effective as the basidiospores require adequate dead wood to become established. Trees and shrubs at field margins could easily be isolated from *Armillaria* species in the same way by cultivated or grass-only cordons.

The pathogenicity of *Armillaria* species has rarely been studied, especially on unusual hosts, largely due to a lack of suitable *in vivo* tests. However, artificial inoculation with *A. mellea* has been successful with woody plants in the field (Bliss 1941; Raabe 1966; Guillaumin and Rykowski 1980; Rykowski 1980, 1981, 1984; Mallett and Hiratsuka 1988; West 1994) or in pots in the greenhouse (Singh 1980a). Strawberry (Fox and Popoola 1990; West 1994) and potato tubers (Gregory 1984, 1985) have also been successfully inoculated with *Armillaria* species.

Most inoculations are carried out with a wooden substrate (Raabe 1962b; Rishbeth 1984), although Plakidas (1941) found that pure cultures of *A. tabescens* gave satisfactory results and Holdenrieder (1987) used individual detached-rhizomorphs. In

addition to wood billets produced cheaply and in quantity by chipping machines, other methods of inoculation could involve the use of starchy-rooted or tuberous hosts such as potatoes, possibly also in chip form. As both of these inocula would need to be inserted in the soil around the crown region of the host they would require either mechanical planting with a tractor-mounted drill or to be shot from a modified gun. In nature, *A. mellea* generally infects hosts through rhizomorphs which spread through the soil from the inoculum source, and additionally or alternatively by direct root-to-root contact. The latter involves mycelial growth directly from the infected root (or inoculated hazel billet) to an adjacent or touching healthy root. In this experiment, no rhizomorphs were observed, indicating that mycelial infection had occurred. Waterlogging in pot experiments can prevent rhizomorph formation (Guillaumin and Leprince 1979) probably due to the low oxygen and high carbon dioxide levels found in the soil (Redfern 1973; Hintikka 1974; Morrison 1976; Rishbeth 1978; Redfern and Filip 1991).

Even though *A. mellea* and *A. ostoyae* are considered primary pathogens on most of their hosts (Raabe 1967; Guillaumin and Pierson 1978; Singh 1980a, b; Singh and Siddu 1989; Morrison *et al.* 1989), Wargo (1980) has argued that host plants often require some stress to predispose them to infection. Nonetheless, all plants suffer some sort of stress and *A. mellea* is itself absent when oxygen is deficient (Redfern 1973; Hintikka 1974; Morrison 1976; Rishbeth 1978; Redfern and Filip 1991). In an integrated control system it is likely that stress could be exerted by the simultaneous application of a foliar herbicide.

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